REMARKS

Claims 8, 10, 11, 16, 26, 28, 29, and 37-51 were pending in the application. Claims 8, 16, 26, 37, 44, 49, and 50 have been amended. Support for the amendment to claims 8, 26, 37, 44, 49, and 50 can be found at page 17, lines 1-3 and page 18, lines 3-20 of the specification. Accordingly, claims 8, 10, 11, 16, 26, 28, 29, and 37-51 are now pending. No new matter has been added.

The specification has also been amended to include a sequence identifier for the described sequence. No new matter has been added.

Amendments to the claims should in no way be construed as an acquiescence to any of the Examiner's rejections and were done solely to expedite the prosecution of the application. Applicants reserve the right to pursue the claims as originally filed in this or a separate application(s).

Sequence identifier

As requested by the Examiner, Applicants have amended the specification to include a sequence identifier for the sequence at page 20 of the specification.

Previous rejection of claims under 35 U.S.C. § 103

Applicants gratefully acknowledge the Examiner's withdrawal of the rejection of the claims under 35 U.S.C. § 103.

Supplemental Information Disclosure Statement

Applicants respectfully request that the Examiner acknowledge the Supplementary Information Disclosure filed on February 3, 2006.

Rejection of claims 8, 10-11, 16, 26, 28-29, 37-42, 44-47, and 49 under 35 U.S.C. § 112, 1st paragraph.

The Examiner has rejected claims 8, 10-11, 16, 26, 28-29, 37-42, 44-47, and 49 under 35 U.S.C. § 112, first paragraph. The Examiner alleges that the phrase "at least 70% biologically active" is not supported in either the claims or specification. Applicants respectfully traverse this rejection.

Applicants point to Figure 9 for support of the phrase "70% biologically active".

Figure 9 provides a graph showing experimental results obtained from mammalian cell cultures incubated at a range of temperatures, wherein incubation temperature (x-axis) is plotted against % dead, *i.e.,* inactive, hLTβR (y-axis). Figure 9 shows about 30% dead, *i.e.,* inactive, hLTβR at an incubation temperature of about 35 ° C, thus providing support for at least 70% biologically active protein.

An Applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, *figures*, diagrams, and formulas that fully set forth the claimed invention. Lockwood v. American Airlines, Inc., 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997). (MPEP 2163.02). In view of the description of the claimed invention described in Figure 9 of the instant specification, Applicants respectfully request the Examiner withdraw the rejection of claims 8, 10-11, 16, 37-42, 44-47, and 49 under 35 U.S.C. § 112, first paragraph.

Rejection of claims 8, 26, 39, 40, 42, 43, and 49 under 35 U.S.C. §102(b) in view of Beutler *et al.*

The Examiner has maintained his rejection of claims 8, 26, 39, 40, 42, 43, and 49 as being anticipated by Beutler *et al.* (U.S. Patent 5, 447,851; hereinafter Beutler) as evidenced by Invitrogen Life Technologies Manual (*Baculodirect™ Baculovirus Expression System,* 2004; Version F: 1-64; hereinafter Invitrogen manual). The Examiner suggests that the purified mammalian cell-derived media described in Beutler is equivalent to Applicants' invention. The Examiner suggests that the process of making the claimed invention includes the same purification steps described in Beutler. Applicants respectfully traverse this rejection.

Claims 8 and 26 have been amended to specify a preparation comprising growth media and at least 70% biologically active receptor-Ig fusion protein and no more than 30% inactive receptor-Ig fusion protein obtained by culturing a mammalian or yeast host cell. Claim 49 has been amended to specify a highly enriched cell culture supernatant comprising growth media and at least 70% biologically active receptor-Ig fusion protein and no more than 30% inactive receptor-Ig fusion protein obtained by culturing a mammalian host cell.

Beutler describes chimeric proteins comprising an extracellular domain of a TNF receptor covalently linked to an IgG molecule and conventional expression of these "TNF chimeras." Beutler describes conventional cell culture, including baculovirus and

mammalian cell expression systems, as well as standard protein chromatography with which to purify the culture media.

Under 35 U.S.C. §102, for a prior art reference to anticipate a claimed invention, the prior art must teach ***each and every element*** of the claimed invention. *Lewmar Marine v. Barient*, 827 F.2d 744, 3 USPQ2d 1766 (Fed. Cir. 1987). Furthermore, "the identical invention must be shown in as complete detail as is contained in the...claim." *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989).

Applicants submit that Beutler does not teach each and every element of amended claims 8, 26, 39, 40, 42, 43, and 49. Each of the claims describes a preparation or a cell culture supernatant comprising growth media and a high yield of biologically active receptor-Ig fusion proteins. Beutler does not teach or suggest a preparation or a cell culture supernatant comprising both growth media and at least 70% biologically active receptor-Ig fusion proteins.

The "low pH elution" taught by Beutler is obtained using a process not required by the claims, and, therefore, not equivalent to the product resulting from the claimed process. Preparations obtained from additional chromatographic purification steps referred to by the Examiner are not equivalent to the claimed preparations or cell culture supernatants which comprise growth media. In fact, the purification steps cited by the Examiner would serve to separate the claimed preparation, as the receptor-Ig fusion protein would be purified from the preparation or cell culture supernatant which also comprises growth media.

In addition, Applicants submit that the Examiner has failed to establish that the "low pH elution" described in Beutler comprises a high yield, *i.e.*, at least 70%, of biologically active receptor-Ig fusion proteins as required by the pending claims. The claimed compositions are obtained by culturing either a mammalian or yeast cell at a low temperature relative to conventional temperatures, a process which does not require additional steps of purification to achieve a preparation comprising at least 70% biologically active molecules. Beutler does not describe any level of biological activity with respect to the described elutants. The preparations taught by Beutler do not anticipate the claimed invention because the cited reference does not teach or suggest any preparation or cell culture supernatant having at least 70% biological activity, which is a required element of the claims.

Importantly, the compositions described in Beutler with respect to insect-cell based protein expression systems are structurally different that the claimed invention. Although different protein expression systems, *i.e.*, insect cells, mammalian cells, and yeast cells, may contain the same gene for expression, the resulting protein preparations obtained from each

system are inherently different and are not structurally equivalent. It is well established in the art that insect cells, mammalian cells, and yeast cells each have different post-translational modifications, particularly with respect to glycosylation patterns. Glycosylation, including differences in N- and O-linked glycosylation, affects the overall structure of the expressed protein. In support of this assertion, Applicants provide the following scientific reviews which describe differences in the various expression systems:

1. Geise *et al.* (1996) *Protein Expression and Purification* 8:271-292 (hereinafter "Geise reference"); and
2. Altmann *et al.* (1999) *Glycoconj J* 16:109-123 (hereinafter "Altmann reference").

The Geise reference describes a comparative study of the expression of a protein (leukemia inhibitory factor (LIF)) using four different mammalian expression systems and the baculovirus expression system. The Geise reference states at page 275, second column, that insect cells do not perform complex type glycosylation, as evidenced by the results shown in Figure 2. Figure 2 shows a comparison of the LIF protein expressed in each system and reveals the differences in glycosylation patterns obtained from the various systems. The Geise reference summarizes the findings of Figure 2 stating "[w]hile hu-LIF produced by CHO, Sp2/0, and MEL cells displayed a broad, but distinct band of approximately 40kDa, hu-LIF derived from baculovirus-infected cells manifested itself as a ladder of discrete bands of smaller size, probably reflecting incomplete glycosylation."

The Altmann reference also provides a review of the use of insect cells for protein expression, particularly with respect to post-translational modification. The Altmann reference concludes the overview by stating at page 117 that "[e]ven though some insect cell lines...exhibit remarkable glycosylation capacity...the 'complex type' N-glycans from insects are very different from the mammalian models." These references provide evidence of the knowledge in the art that receptor-Ig fusion proteins expressed in mammalian and insect cells are not structurally identical due to inherent differences in post-translational modifications in the expression systems.

As described in Applicants' previous response, Beutler describes protein expression using mammalian and yeast expression systems which produce products which are not equivalent to the claimed invention. Beutler teaches conventional culturing techniques and does not teach or suggest lowering the culturing temperature to achieve a high yield of biologically active receptor-Ig fusion proteins. As stated above, the Examiner has provided no evidence that the mammalian cells referred to in Beutler would be cultured at anything other than the standard temperature, or that the resulting culture supernatants would contain a

high yield of biologically active molecules. Applicants provide data in the instant specification which describes the activity differences between the standard temperature and the low temperature method preparations claimed herein. Table II at page 25 of the specification describes the percentage of inactive receptor-Ig fusion protein in cultures obtained from CHO cells. Table II illustrates that a preparation obtained by culturing the mammalian CHO cells at the standard culture temperature of 37 °C comprises 50% inactive LT-β-R-Ig protein. Table II shows in contrast that a preparation obtained by culturing mammalian CHO cells at a temperature below the standard culture temperature, *i.e.*, 32 °C or 28 °C, comprises an improved 17% and 10% inactive LT-β-R-Ig protein, respectively. In sum, the instant specification provides a controlled experiment which contrasts preparations obtained using conventional standards (like those described in Beutler with respect to yeast and mammalian expression) versus those made at temperatures lower than the conventional temperature (like those claimed in the instant invention).

In conclusion, to serve as an anticipation rejection, a reference must disclose each and every limitation of the claimed invention. Beutler fails to teach the claimed preparation and cell culture supernatant obtained by culturing either mammalian or yeast cells at a lower temperature relative to conventional temperatures, comprising growth media and at least 70% biologically active receptor-Ig fusion proteins. The baculovirus preparations described by Beutler are structurally different than the claimed invention due to inherent differences between the insect cell expression system and the claimed yeast and mammalian systems. In view of these differences and the amendments to the claims, Beutler fails to anticipate the claimed invention. Applicants respectfully request that the rejection of claims 8, 26, 39, 40, 42, 43, and 49 under 35 U.S.C. § 102(b) be withdrawn.

Rejection of claims 8, 11, 26, 29, 37, 38-40, 42-43, and 49 under 35 U.S.C. §102(b) in view of Ashkenazi *et al.*

The Examiner has maintained the rejection of claims 8, 11, 26, 29, 37, 38-40, 42, 43, and 49 as being anticipated by Ashkenazi *et al.* (WO 98/25967; hereinafter Ashkenazi) as evidenced by Invitrogen Life Technologies Manual (*Baculodirect™ Baculovirus Expression System,* 2004; Version F: 1-64; hereinafter Invitrogen manual). The Examiner states that Ashkenazi teaches "numerous means of purification using 'conventional' chromatographic methods" which one of ordinary skill in the art would use to arrive at the claimed invention. Applicants respectfully traverse this rejection.

The claims have been amended to specify a preparation comprising growth media and at least 70% biologically active receptor-Ig fusion protein and no more than 30% inactive receptor-Ig fusion protein obtained by culturing a mammalian or yeast host cell. In addition, claim 49 is directed to a highly enriched cell culture supernatant comprising growth media and at least 70% biologically active receptor-Ig fusion protein and no more than 30% inactive receptor-Ig fusion protein obtained by culturing a mammalian host cell.

Ashkenazi describes HVEM polypeptides, including nucleic acids encoding said and chimeric proteins. With respect to the expression of HVEM polypeptides, Ashkenazi describes a variety of potential host cells for expression, including insect cells, plant cells, and mammalian cells. Following culturing of the cells, Ashkenazi describes purification techniques, which are referred to by the Examiner as being equivalent to Applicants' invention.

The purified elutants described by Ashkenazi are not equivalent to the high yield preparation and highly enriched cell culture supernatant described in amended claims 8, 11, 26, 29, 37, 38-40, 42, 43, and 49. The preparations and cell culture supernatant described in the claimed invention comprise growth media and minimal amounts of the inactive receptor-Ig fusion protein, *i.e.*, at least 70% biologically active receptor-Ig fusion proteins. The purified preparation described in Ashkenazi is not a high yield preparation or a highly enriched cell culture supernatant comprising growth media and receptor-Ig fusion proteins. Indeed, the purified preparation referred to in Ashkenazi results from multiple purification steps which isolates the protein of interest from the cell culture supernatant.

Ashkenazi also fails to teach a high yield preparation and highly enriched cell culture supernatant having at least 70% biologically active receptor-Ig fusions. Ashkenazi does not teach or suggest deviating from conventional protein expression systems, as the reference does not teach or suggest altering culturing temperatures to improve the percentage of overall active molecules. Furthermore, as described above, preparations obtained from insect cells are structurally distinct from the claimed invention, which is obtained from mammalian and yeast host cells.

In conclusion, to serve as an anticipation rejection, a reference must disclose each and every limitation of the claimed invention. Ashkenazi fails to teach the claimed preparation and cell culture supernatant obtained by culturing either mammalian or yeast cells at a lower temperature relative to conventional temperatures, comprising growth media and at least 70% biologically active receptor-Ig fusion proteins. Applicants respectfully request that the rejection of claims 8, 26, 39, 40, 42, 43, and 49 under 35 U.S.C. § 102(b) be withdrawn.

Rejection of claims 8, 10, 16, 26, 28, and 39-51 under 35 U.S.C. §102(b) in view of Degli-Esposti *et al.*

The Examiner has rejected claims 8, 10, 16, 26, 28, and 39-51 as being anticipated by Degli-Esposti *et al.* (hereinafter Degli-Esposti). The Examiner alleges that the chromatographic purification of secreted LTβR-Fc proteins described in Degli-Esposti anticipates the claimed invention. Applicant respectfully traverses this rejection.

The Degli-Esposti reference describes expression of LT-β-R-Fc in mammalian CVI/EBNA cells. Degli-Esposti also describes expression of an Ig-fusion protein using standard culturing techniques known in the art. The Degli-Esposti reference does not teach or suggest a high yield preparation or a highly enriched cell culture supernatant comprising growth media and at least 70% biologically active receptor-Ig fusion proteins, as required in amended claims 8, 10, 26, 28, 39, 40, and 42-51. The Degli-Esposti reference also does not teach or suggest all of the limitations of claims 16 and 41, as it does not teach or suggest a pharmaceutical preparation obtained by culturing mammalian cells at a reduced temperature to achieve a cell culture supernatant comprising at least 70% biologically active LTβR -Ig-fusion proteins, recovering the LT-β-R-Ig-fusion proteins, and combining the LTβR-Ig-fusion proteins with a pharmaceutically acceptable carrier.

The Examiner suggests that the biological activity of the composition described in the Degli-Esposti reference is at least 70% biologically active because it was obtained using affinity chromatography. Indeed, Applicants' claims make no mention of affinity chromatography to obtain a preparation or cell culture supernatant having at least 70% biologically active receptor-Ig-fusion protein. Thus, Applicants submit that the resulting product obtained from chromatography is not equivalent to the claimed high yield preparation and cell culture supernatant comprising growth media. Furthermore, the resulting product obtained from chromatography is not equivalent to the claimed pharmaceutical preparation. Applicants note that the chromatography techniques described in the instant specification, referred to by the Examiner, provide a means of assaying the active and inactive percentage of receptor-Ig fusion proteins within the claimed preparations obtained using the low temperature culturing method. These chromatographic techniques, however, are not recited as a step in obtaining the claimed compositions.

In view of the amendments to the claims and the above-mentioned comments regarding the failure of the Degli-Esposti reference to teach each and every limitation of the

claims as required under 35 USC §102(b), Applicants respectfully request that the rejection be withdrawn.

CONCLUSION

In view of the foregoing comments, reconsideration of the rejections and allowance of all pending claims is respectfully requested.

If a telephone conversation with Applicants' Attorney would expedite the prosecution of the above-identified application, the examiner is urged to call Applicants' Attorney at (617) 227-7400.

Respectfully submitted,

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Dated: **August 7, 2006**